(6)

botany

UNIVERSITY OF TORONTO · DEPARTMENT OF BOTANY · TORONTO, ONT. M5S 1A1

February 1, 1980

PLANT DEVELOPMENT WORKSHOP

SPRING, 1980

Dear

You and your colleagues are invited to attend the 6th Plant Development Workshop which will be held at the Botany Department of the University of Toronto on Saturday, March 29, 1980.

Our tentative schedule is:

9:30 - 10:00 a.m.	Coffee and registration
10:00 - 12:00 noon	Contributed papers (15 minutes each)
12:00 - 2:00 p.m.	Buffet lunch and discussion of contributed posters
2:00 - 4:00 p.m.	Contributed papers and/or discussion.

We will plan to restrict presentations to 15 minutes in order to allow ample time for discussion. I would appreciate receiving your suggestions for general discussion topics as well.

Please send titles and abstracts (maximum 250 words) to me before March 14, 1980. Could you also let me know how many people will be coming with you so that we can plan for lunch.

We hope that you are able to attend.

Sincerely,

Nancy G. Dengler
Associate Professor and
Associate Chairman

NGD:cdj

Dr. Nancy G. Dengler
Department of Botany
University of Toronto
Toronto, Ontario M5S 1A1

(416)-978-2018

March 29, 1980

botany

UNIVERSITY OF TORONTO · DEPARTMENT OF BOTANY · TORONTO, ONT. M5S 1A1

Second Notice

PLANT DEVELOPMENT WORKSHOP

March 29, 1980

TENTATIVE WORKSHOP SCHEDULE:

9:30 a.m. - 10:00 a.m.	Coffee, Room 207
10:00 a.m. - 12:00 noon	Presentation of papers, Room 7
12:00 noon - 1:30 p.m.	Lunch and discussion of posters
1:30 p.m. - 3:30 p.m.	Proposal for Fall, 1980, workshop Presentation of papers
3:30 p.m. - 4:30 p.m.	Wrap-up and refreshments

I'm enclosing a map of the St. George Campus of the University of Toronto and some suggestions for finding parking.

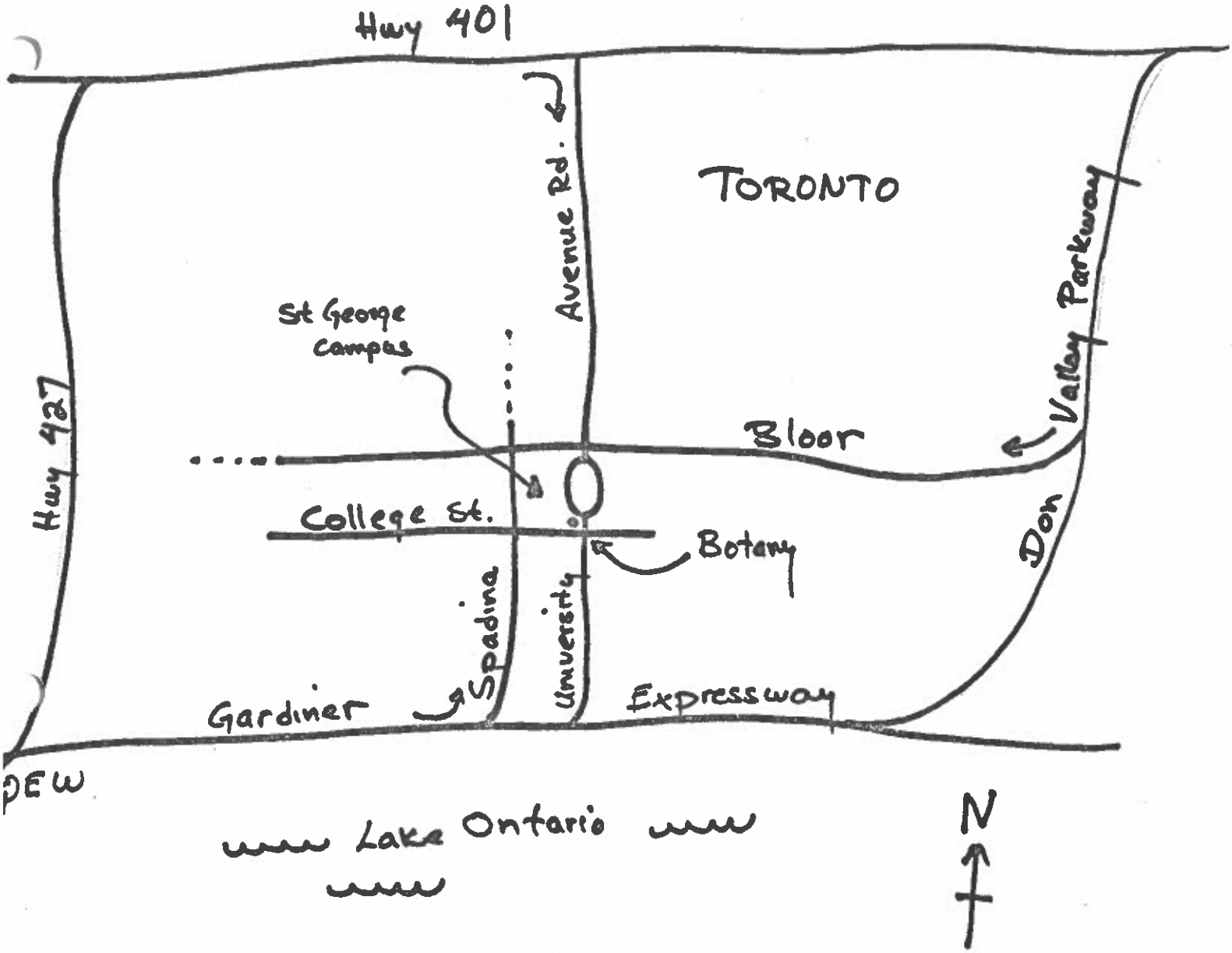
If you haven't already done so, please let me know (by Wednesday, March 24) how many people are coming from your laboratory.

I'm looking forward to seeing you!

Nancy

Nancy G. Dengler
(416) 978-2018)

Map of St. George Campus, University of Toronto, location #6



Parking:

- Courtyard of Botany Bldg (enter from College St.)
- metered parking on College St.
- Taddle Creek Rd.
- Kings College Rd. and Circle

see map of campus

(6)

THE 6TH PLANT DEVELOPMENT WORKSHOP

March 29, 1980

Department of Botany, University of Toronto

9:30 - 10:00 Registration, coffee and donuts, Room 207C

10:00 - 12:00 Morning Session, Room 7

1. The accommodation growth of Ranunculus scleratus petioles. Ananda Samarakoon, Ingrid Boesel and Roger Horton, Dept. of Botany and Genetics, University of Guelph.

2. Effects of excision of stock and scion organs on the formation of the graft union in Coleus. Frederick L. Stoddard, Depts. of Biology, University of Ottawa and Carleton University.

3. The relationship between meristem architecture and leaf and internode shape in Zea mays. R.I. Greyson, D.B. Walden, W. Smith and M. Moyles. Department of Plant Sciences, University of Western Ontario.

4. Anatomy of young sporophytes of the kelp, Macrocystis pyrifera. Douglas Grenville, H. Barrales, R.L. Peterson and J.G. Gerrath. Department of Botany and Genetics, University of Guelph.

5. Cell wall of Charophytes. T. Sawa, Dept. of Botany, University of Toronto.

6. Release of Rhizobium from the infection threads in root nodules. Laurel McIntyre and William Newcomb, Biology Department, Queen's University.

12:00 - 1:15 Lunch, Room 207C

1:15 - 2:45 Afternoon Session, Room 7

Brief discussion of Fall, 1980, workshop - John N.A. Lott.

7. Structure and composition of the protein bodies in a number of Umbelliferous seeds. Ernest Sptizer and John N.A. Lott, Dept. of Biology, McMaster University.

8. Analysis of plant cells using soft X-ray lithography. P.C. Cheng, F. Wendy, J.W. McGowan, R.I. Greyson and D.B. Walden, University of Western Ontario.

9. Cell cycle kinetics in meristems of roots grown in different amounts of water. J.E. Thomas, Biology Department, McMaster University.

10. Inhibition of necleolar, nuclear and cell growth by methylxanthines. D. Davidson, Biology Dept., McMaster University.

11. Caffeine: The drug. S.W. Armstrong, Biology Dept., McMaster University.

3:00 - 4:00 Film and discussion: Light microscopy and cinemicrography of living sieve tube elements, mostly in Heracleum mantegazzianum L. G.F. Barclay, Dept. of Biology, Waterloo University.

4:00 - 5:00 Wrap-up and refreshments.

THE 6TH PLANT DEVELOPMENT WORKSHOP

March 29, 1980

Department of Botany, University of Toronto

ABSTRACTS

1. The accommodation growth of Ranunculus scleratus petioles. Ananda Samarakoon Ingrid Boesel and Roger Horton, Dept. of Botany and Genetics, University of Guelph.

The petioles of the semi-aquatic buttercup Ranunculus scleratus elongate rapidly when submerged, allowing accommodation to changing water levels. Ethylene accumulates in the leaf tissue during submergence causing rapid cell elongation in the petioles.

Petioles of excised leaves elongate in response to ethylene or IAA. Ag^{+} ions inhibit the response to ethylene but not that due to auxin. Submergence in dilute solutions of Co^{++} ions inhibits accommodation growth, but the tissue remains responsive to added ethylene. Treatment with anti-auxin compounds inhibits the ethylene response. Removal of the blade causes a marked reduction in the response of petioles to ethylene. However, when isolated petioles are treated simulatneously with ethylene and IAA a synergistic elongation response is produced and this approaches the elongation of bladed petioles under submergence. Petiole elongation is therefore regulated by ethylene in the presence of auxin. CO_2 and GA either individually or in combination with ethylene or IAA, have no significant effect on this rapid petiole growth.

Ethylene is derived largely from the blade tissue, and its production is enhanced by treatment with 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene. ACC accumulates in leaves during submergence.

The petiole has an extensive aerenchymatous tissue and the blade is amphistomatous.

2. Effects of excision of stock and scion organs on the formation of the graft union in Coleus. Frederick L. Stoddard, Depts. of Biology, University of Ottawa and Carleton University.

Effects of removal of leaves, shoots and roots on the formation of the graft union in Coleus stems are investigated. The grafting process is separable into the following components: callusing of the cut surfaces, necrotic layer removal, cohesion of the graft partners, bridging of the graft by xylem and phloem, formation of a graft-bridging cambium. Isolated internode grafts cohere without apparent participation of the other four components.

Quantitative data are presented for the production of vascular tissue bridging the graft. An apparent acropetal flow of xylem-inducing stimuli, basipetally moving stimuli from immature stem tissue, apical and auxiliary shoot tips, and leaves that are at least half

...cont'd

mature size contribute, respectively, 8.4%, 9.5%, 10.1% and 36.1% of the amount of xylem in grafts of otherwise intact plants. The remaining 35.9% of xylem-inducing stimuli may be from synergistic effects of the scion organs. The scion organs nearest the graft contribute more stimuli than do those more distant. Leaves and shoots on the stock have no effect on the graft. The ratio of graft-bridging strands of phloem and xylem is not significantly changed by any treatment, and averages 0.226:1, compared to 2:20:1 in intact internodes.

3. The relationship between meristem architecture and leaf and internode shape in Zea mays. R.I. Greyson, D.B. Walden, W. Smith and M. Moyles. Department of Plant Sciences, University of Western Ontario.

We have accumulated data which documents the obvious but not carefully studied leaf heteroblasty of corn (Zea mays). Leaf size and shape, at maturity, vary along the stem in regular, cultivar-related patterns when plotted as double-log displays of leaf length and width. These curves, possessing distinct inflection points which coincide on the plant with the position of the female inflorescence, vary depending upon the cultivar. Similar length/width plots for internode data also document heteroblasty. Graphical summaries of data from freshly dissected meristems at all plastochron stages from germination to tassel initiation document that meristem height and diameter both increase throughout the period of leaf initiation. No parallel for the inflecting curves of leaf shape is detected in the architectural development of the meristem. We conclude therefore that mature leaf and internode shapes are not significantly determined by the shape or size of the meristem from which they arise.

4. Anatomy of young sporophytes of the kelp, Macrocystis pyrifera. Douglas Grenville, H. Barrales, R.L. Peterson and J.G. Gerrath. Department of Botany and Genetics, University of Guelph.

Although there has been considerable research on the ecology and physiology of Macrocystis, there has been less attention paid to the structure and development of this giant kelp. Earlier workers concentrated mainly on the mature plant, particularly the cells involved in photosynthate transport. Preliminary observations of very young sporophytes collected in the kelp beds off Patagonia, Argentina, suggest some interesting developmental problems. Mucilage canal formation, cortical cell differentiation, trumpet hyphae initiation and development, and the mechansim by which blades are sub-divided during growth have not been studied in detail. Blades and stipes have been examined by a number of techniques, including light and electron microscopy, in an attempt to understand some of these developmental processes in young sporophytes.

5. Cell wall of Charophytes. T. Sawa, Dept. of Botany, University of Toronto.

The inner surface of the internode cell wall of charophytes can be classified into three types, smooth, papilate and "pitted." They characterize the walls of the genera Nitella, Chara and Talypella respectively.

Although our preliminary study concludes that the feature is a new criterion to discuss the controversial taxonomy of these unique aquatic macrophytes, the identity of the feature is still under investigation and only conjectural arguments can be presented at the present moment.

6. Release of Rhizobium from the infection threads in root nodules. Laurel McIntyre and William Newcomb, Biology Dept., Queen's University.

The morphogenesis of root nodules involves an intricate and intimate interrelationship between the eukaryotic host- the legume- and the micro- symbiont- a prokaryotic bacterium belonging to the genus Rhizobium. During the development of a nodule a free living rhizobum cell penetrates a specific cell- the root hair - and forms in co-operation with the host an infection thread. The infection thread subsequently ramifies and invades many of the root cortical cells and their derivatives. Eventually the rhizobia escape from the infection thread into the cytoplasm where the rhizobia are always surrounded by a peribacteroid membrane (formerly called membrane envelope). In pea, lupin, and soybean nodules numerous workers have shown that the peribacteroid membrane is initially derived from the host plasma membrane. However, some workers have suggested other origins, such as out-blebbing of the nuclear envelope in mung bean nodules. In this report evidence will be presented to demonstrate that the peribacteroid membrane is initially derived from the host plasma membrane in both mung bean and alfalfa root nodules. In addition, differences between the infection threads of various species will be discussed because these create difficulties in the interpretation of micrographs.

7. Structure and composition of the protein bodies in a number of Umbelliferous seeds. Ernest Spitzer and John N.A. Lott, Dept. of Biology, McMaster University.

Protein bodies are generally the major sites of protein and mineral storage in seeds. Protein bodies have a proteinaceous matrix, often contain globoid regions, and may have a protein crystalloid component. Still other protein bodies contain druse crystals thought to be calcium oxalate. Druse crystal containing protein bodies are widespread in the Umbelliferae. TEM, SEM and light microscopy studies of protein bodies in seeds from nine umbelliferous species (carrot, caraway, anise, celery, dill, parsnip, parsley, fennel and chervil) were undertaken.

...cont'd

Protein bodies of two structural types were found in the umbelliferous seeds studied. One type, which was widely distributed in embryo and endosperm regions consisted of proteinaceous matrix surrounding a number of globoid crystals of various sizes. A second protein body type consisted of proteinaceous matrix and a druse crystal. These druse crystal containing protein bodies were found only in cells of the endosperm. In endosperm the two types of protein bodies were never found in the same cell. There was no special distribution pattern observable between cells containing protein bodies with globoid crystals and cells containing protein bodies with druse crystals. Energy dispersive x-ray analysis was used to obtain elemental composition of globoid and druse crystals in embryo and endosperm regions. Additional methods including acid solubility, incineration and infra-red analysis were used to determine the chemical nature of the druse crystals. The results indicate that the druse crystals are composed of calcium oxalate.

8. Analysis of plant cells using soft X-ray lithography. P.C. Cheng, F. Wendy, J.W. McGowan, R.I. Greyson and D.B. Walden, Dept. of Plant Sciences and Dept. of Physics, University of Western Ontario.

Following the discovery of x-rays at the turn of the century, biologists have attempted to carry out high resolution soft x-ray microscopic study of cells. The lack of quality focusing elements and intense x-ray sources have made it impossible to image the cell while intensity, resolution of the recording film and the tunability of the x-ray source have limited contact microscopic studies to dimensions larger than 1 μm and virtually eliminated the possibility of using soft x-rays for microchemical analysis of either wet or dry biological samples. Since the mid-seventies the situation has begun to change. High reflectivity x-ray mirrors and Fresnell zone-focusing elements are now under development which will eventually make transmission microscopy possible. Furthermore, very intense pulse sources and tunable synchrotron radiation sources, as well as high resolution film (x-ray resists), which have been developed for the microcircuit industry, now make it possible to obtain contact prints with resolutions better than 10 nm.

In this preliminary study we have applied contact microscopy to 0.3 μm sections of corn flowers and anther tissues of Caltha palustris L. fixed in Cheng's fixative, post-fixed in OsO_4 and embedded in Spurr's medium. The sections were mounted on 100 nm Si_3N_4 windows developed for soft x-ray microscopic work by scientists at IBM, Yorktown Heights, N.Y. The samples were mounted in a spring-loaded holder with the sections in intimate contact with the polymethyl methacrylate x-ray resists (PMMA). Two x-ray energies (4.48 nm C-Kα and 2.74 nm Ti-Lα) were used to replicate the samples. These photo energies are above and below the Ca-L absorption edges and by taking the difference between the two replicas one hopes to determine the distribution of calcium within the sample.

....cont'd

Development of the x-ray resists in a 1:1 (v/v) solution of methyl isobutyl ketone and isopropanol for five minutes gives topographic replicas of the sample which accurately reflect the differential absorption of the sample at the two x-ray energies used. The developed resists were then coated with a thin layer AuPd by the method of ionic sputtering to allow viewing with the SEM at 20 kV in the secondary emission mode and by interference light mocroscopy. One is able to correlate much of the structure observed in replica with the structures known in the tissues of Caltha palustris L. and Zea mays L. In particular one can observe structures such as nucleus and nucleolus, lipid bodies, exine and Ubisch bodies, etc. which can be correlated to those seen by TEM and LM techniques. Although there is a difference between the two replicas above and below the L absorption edges, it is still too early to tell if the difference is due only to the presence of calcium in the cell or other elements. Further experiments are presently in progress.

9. Cell cycle kinetics in meristems of roots grown in different amounts of water. J.E. Thomas, Biology Dept., McMaster University.

Roots grown in tanks of water elongate at a slower rate and have a lower mean M.I. than roots grown in vermiculite-perlite (V/P) moistened with 40 ml water/100 ml V/P. In order to compare cell cycle kinetics in the two systems roots grown under both conditions were treated with ^{3}H-TdR for 1 hr. and fixed 0 to 31 hr. after treatment. Roots grown in tanks of water had a mean cycle time (T_C) of 23.4 hr. (T_{G1} = 6.1 hr., T_{G2} = 5.5 hr., T_S = 9.6 hr., T_M = 2.3 hr.) while roots grown in V/P had a shorter mean cycle time, T_C = 17 hr. (T_{G1} = 5.6 hr., T_{G2} = 4.0 hr., T_S = 5.0 hr., T_M = 2.4 hr.). Most of the difference in cycle times was due to a disproportionate increase in the duration of S for water grown roots while the duration of G_1 and G_2 showed relatively smaller changes. This difference in T_S was coupled with an increase in mean labelling index (L.I.) in water grown roots over that seen in V/P grown roots, i.e., 31.0 ± 8.6 cf. 25.9± 6.5. Changes in the proportions of fast, slow and non-cycling cells also occurred; i.e., in V/P grown roots there were 83.2% fast, 11.4% slow and 5.4% non-cycling cells while in tanks of water there were 52.9% fast, 22.7% slow and 24.4% non-cycling cells. These results show 1) variation both in cycle duration and phase duration within the cell cycle for roots grown in different amounts of water and 2) that the response to water affects more than 1 regulatory event in the cell cycle.

10. Inhibition of necleolar, nuclear and cell growth by methylxanthines. D. Davidson, Biology Dept., McMaster University.

It has previously been reported that: 1) methylxanthines, e.g. caffeine and 3-isobutyl-1-methylxanthine (IBMX), induce the formation of binucleate cells; 2) the two sister nuclei in a binucleate cell differ in size, i.e.,show differential growth; 3) nuclei undergo a contraction in the first 1-3 hours of interphase. Associated with the failure, in early G_1, of nuclei and cells to grow it has been found than nucleoli do not undergo nucleogenesis at normal rates. It may be, in fact, the inhibition of nucleolar function (i.e. transcription of rDNA) that is responsible for the slow growth of nuclei and cells. Zea mays roots were treated with IBMX, 8-EOC (8-ethoxycaffeine) or colchicine for 2 hours: nucleolar and nuclear volumes and cell areas were determined immediately after treatment and after 6 hours recovery. A binucleate cell (2n + 2n) or a tetraploid cell (4n) in G_1, both contain the 4C DNA content and are functionally equivalent to a 2n nucleus in G_2 (which also has a 4C DNA content). Total nucleolar volume per binucleate cell after 2 hours methylxanthine treatment was 13 to 18 μm^3, cf. 31 μm^3 in normal interphase nuclei. After 6 hours recovery their mean volumes were still only 19 to 22 μm^3. Yet 6 hours after colchicine treatment, mean nucleolar volume was 37.7 μm^3. Parallel inhibitions of nuclear and cell growth were also found. It is concluded that treatment with a methylxanthine induces severe disturbances of the ability of chromatin to support normal levels of gene function. It is probably these effects that are responsible for the overall inhibition of cellular growth by methlxanthines.

11. Caffeine: The drug. S.W. Armstrong, Biology Dept., McMaster University.

Average consumption of caffeine in N. America is ∿200 mg/day. Significant biological effects can be induced by ingestion of 100 mg/day. While the physiological effects of caffeine are known, the mechanism(s) by which it exerts ifs effects is unknown. At the cellular level, caffeine and other methylxanthines, e.g. theophylline, theobromine, inhibit enzyme activity (3i,ii), depress DNA, RNA and protein synthesis (3iii, iv, 4ii, iii). The effects on post-replication systems may lead to chromosome breakage and/or mutation. Caffeine has recently been used to control cell proliferation and differentiation.

Results from this lab have also shown that pulse treatments with caffeine: 1) alter cell cycle parameters, mainly by prolonging G_1; 2) depress RNA synthesis and uptake of preformed RNA; 3) inhibit post-mitotic nucleogenesis; 4) reduce growth of nucleoli, nuclei and cells; 5) induce polysaccharide formation.

A summary of the changes induced by methylxanthines is give below.

1. Structural effects
   - i. prevents fusion of vesicles at presumptive cell plant in plants: gives binucleate cells
   - ii. prevent cleavate furrow formation in animals
   - iii. induces microtubule dissociation: differential effect on cortical MTs associated with 2° wall thickening
   - iv. modifies membranes: effect on muscle action potential and on precursor uptake by cells

... cont'd

2. Physiological effects (on organisms)
   i, marked effect on cardio-vascular system
   ii. quickens flow of thought and enhances association of ideas.
3. Physiological effects on cells
   i. inhibits PPDE activity (phophodiesterase: cAMP)
   ii. inhibits poly (ADP-ribose) polymerase
   iii. depression of RNA and protein synthesis
   iv. inhibits DNA synthesis and increases number of DNA replicon initiation sites
   v. arrests normal cells in G_1 (i.e. induces G_0 state): transformed cells not affected (cf. 1. iv)
   vi. delays progresses through G_2 and reduces mitotic index
   vii. induces vegetalization in sea urchin embryos
   viii. induces or enhances process of differentiation
4. Mutagenic and clastogenic effects
   i. induces chromosome breakage in some systems
   ii. inhibits post-replication repair in some systems
   iii. inhibits post-treatment repair of radiation and chemical mutagen induced lesion (may be related to 3.ii.)

12. Light microscopy and cinemicography of living sieve tube elements, mostly in He acleum mantegazzianum L. G.F. Barclay, Dept. of B ology, University of Waterloo.

Sieve elements in isolated phloem bundles of Heracleum mantegazzianum, H. sphondylium, Nymphoides peltata, Cucumis sativus, and Apium graveolens were studied by Nomarski Differential Interference-Contrast microscopy, and a 16mm cine film was made of them.

Phase contrast and ordinary bright field optics were unsuitable for observation of sieve elements because they gave rise to misleading optical artifacts. Some of the "transcellular strands" reported by previous workers to be present in sieve elements viewed with such optics were probably optical artifacts. No transcellular strands were seen in Heracleum sieve elements viewed with Nomarski optics.

Sieve element plastids in isolated phloem bundles were usually burst, indicating that the sieve elements were damaged and unsuitable for translocation study. Plastids which fortuitously remained intact in isolated bundles of Heracleum were induced to break down by treatment with Lugol's iodine. Burst plastids released starch grains and other particles into the sieve element lumina. These particles were probably the same as "marker particles" reported by previous workers in supposedly undamaged sieve elements of Heracleum. Analysis of particle displacement indicated that a report by previous workers that particle motion is sieve elements was greater than predicted by the Einstein equation for Brownian motion, and that it was under physiological control, was probably based on an unreliable method of analysis.

P-protein was present in some sieve elements of Heracleum, but it occurred too rarely and its arrangement was too diverse to make useful speculations about possible functions of P-protein in facilitating translocation in this plant.